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Fumigant Toxicity of Citruspeel Oils against Adult and Immature Stages of Storage Insect Pests

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Abstract: The biological action of citruspeel oils was shown to depend on a strong fumigant action. Bioassays conducted in air-tight glass chambers showed that all the six citrus oils tested had vapour toxicity towards adults of *Callosobruchus maculatus* F., *Sitophilus zeamais* Motsch. and *Dermestes maculatus* Deg. The 24-h LC_{50} value of limepeel oil (a typical citrus oil) vapour against *C. Maculatus* was 7·99 μl litre^{-1} which made it 1·5 and 1·6 times less toxic against the smaller *S. zeamais* and the larger *D. maculatus* adult insects.

When immature stages were fumigated, limepeel oil vapour had 24-h LC_{50} values of 7·8 and 21·5 μl litre^{-1} against eggs of *C. maculatus* and *D. maculatus* respectively, and 9·1, 17·8 and 23·1, 23·9 μl litre^{-1} against early larvae, pupae of *C. maculatus* and late larvae, pupae of *D. maculatus* respectively. X-ray studies showed that fumigated *C. maculatus* larvae within cowpea grains died immediately without further development. The bioactivities of five other citruspeel oils were similar to that of limepeel oil.

Bioassays showed that sorption of citruspeel oil fumes occurred in the presence of grains or strips of dried fish, and that this tended to reduce the amount available for fumigant action outside the materials. The problems presented by sorption may hinder the development of citrus oils into practical fumigants for large-scale treatments of stored commodities.

Key words: fumigant, toxicity, citruspeel oils, adults, immature stages, insect pests

1 INTRODUCTION

Many vegetable oils have been screened for use in preventing post-harvest loses due to insects.[1–3] Most of such work has related to the contact toxicity of the oils against eggs of insect pests. In the first paper in this series the author established that a more efficient way to use citruspeel oils to control insects would be as a fumigant in relatively enclosed or airtight systems. In contrast, fixed vegetable oils like groundnut oil, which do not have volatile components, showed contact activity against eggs with strong residual properties.[2] In Nigeria, merchants rub groundnut or other fixed vegetable oils onto dried fish for protective or cosmetic reasons[3] and in some parts of the country orange peels (containing volatile oils) are burned at night to drive off mosquitoes.[4] However, no report known to the author has described the use of citruspeel oils as fumigants in traditional farms or research laboratories. Therefore, having demonstrated in the first report of this series the greater importance of fumigant toxicity of citruspeel oils over their previously emphasised, but incidental, contact toxicity, this paper reports investigation of the vapour activity of several citrus oils against adults of three different storage pest species. In addition, this work examines the fumigant toxicity of the citrus oils

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against immature stages of storage insects, an evaluation that has not previously been done. The work also includes preliminary trials to examine the influence of sorption on the demonstrated biological activity of the oils within glass chambers.

2 EXPERIMENTAL METHODS

2.1 Test compounds

Industrially extracted citruspeel oils (lime, orange, mandarin, tangerine, grapefruit and lemon peel) were obtained from Zimmermann Hobbs Ltd, UK.

2.2 Insect cultures

Stock cultures of strains of *Callosobruchus maculatus* F., *Sitophilus zeamais* Motsch. and *Dermestes maculatus* Deg. which had been cultured in the absence of insecticides for several years were obtained and cultured as described previously.[5]

All insect cultures were kept at 28°C and 70% RH under constant red light. All bioassays were conducted under these same conditions of temperature, humidity and light.

2.3 General bioassay techniques adopted in fumigation experiments

2.3.1 Fumigation process

The fumigant action of citruspeel oils was assessed in airtight Kilner jars (500 ml) sealed with a screw ring holding a glass lid onto a rubber washer covered with aluminium foil to prevent any reaction with test compounds. This system was adopted because of its simplicity and ease of operation.[6] The test oils were dispensed onto a 3-cm diameter Whatman No. 1 filter paper, as preliminary experiments showed that all test compounds could readily evaporate from the filter paper when this was suspended on a string within the glass chamber in the bioassay room (28°C, 70% RH). The oils were applied to the chamber after addition of the test insects (usually 15 per chamber), the stopper being quickly replaced to minimise loss of volatiles. Insects were confined to the bottom third of the chamber by coating the sides with Fluon, and food was not provided during fumigation. Except where otherwise stated fumigation was for 24 h, at the end of which time the chambers were opened and mortality assessed. Glass chambers were washed clean with water and dried in an oven at 34°C before re-use.

2.3.2 Method of mortality assessment

Insects were taken as dead if they (mobile stages) did not move away when touched gently or tilted with an entomological forcep.

2.4 Effect of binding of oils on fumigation toxicity

The following factors were investigated:

(a) *Treatment surface:* Fifteen adult *C. maculatus* were fumigated with 6 μl limepeel oil per litre with (i) oil deposited on the glass surface (microscope slide) held above Fluon mark or (ii) with oil deposited on the filter paper, and suspended within the glass chamber. All treatments were replicated four times and at the end of fumigation period of 24 h, mortality was assessed.

(b) *Volume of oil applied:* Mixtures (3 + 2 by volume) of (+)-limonene or citronellal (two toxic citrus oil components) were formulated with the non-volatile, non-toxic oil, *n*-decyl alcohol. Fifteen *C. maculatus* adults (mixed sexes, one to two days old) per chamber were fumigated (see Section 2·3) with (i) 3 μl of pure (+)-limonene or citronellal, or (ii) 5 μl of (+)-limonene + *n*-decyl alcohol, or citronellal + *n*-decyl alcohol mixtures (maintaining the volume (3 μl) of toxic oils constant while increasing the overall oil volume by 2 μl of non-toxic oil in the mixtures). All treatments were replicated four times and mortality assessed immediately at the end of 24 h of fumigation.

2.5 Fumigant action of citruspeel oil against adult and immature stages of test insects

2.5.1 Tests with Callosobruchus maculatus

(a) *Adults:* Fifteen one- to two-day-old individually weighed adults (mixed sexes) were fumigated as described above. All citrus oils were tested at concentrations between 6 and 10 μl litre^{-1} for each oil (replicated twice). Mortality was assessed at the end of 24 h of fumigation.

(b) *Eggs:* In post-oviposition treatments, untreated cowpeas harbouring two- to three-day-old eggs on cowpeas were fumigated for 24 h with citrus oils at rates from 4 to 16 μl litre^{-1}. Five egg-infested grains were fumigated per chamber and all treatments were replicated two or three times. The egg-infested seeds (three to five eggs per seed) were prepared by confining clean batches of cowpea grains (40) with sixteen, zero- to one-day-old adults (eight females, eight males) for 24 h. Earlier trials[2] showed that this mode of infestation resulted in an even spread of eggs on 90% of grains. Egg mortality was assessed by counting unhatched eggs (grey dome-shaped, oval-based structures, with or without a dark spot depicting dead larva) under a binocular microscope at x12·5), 12 days after fumigation.

(c) *Larvae/pupae:* Five cowpea grains pre-infested with early stage larvae (11- to 12-day-old) or late-stage larvae/pupae (20- to 21-day-old) were fumigated with limepeel, orangepeel or tangerine peel oils at rates of 6–28 μl litre^{-1} (early larvae) and 12–32 μl litre^{-1} (late

stage larvae/pupae) for 24 h. The larvae-infested seeds (three to five larvae per seed) were prepared by obtaining eggs on clean cowpea seeds as described in Section 2.5.1b. Each hatched egg (hatching occurs four to seven days after oviposition) was taken as a live larva within the grain (in preliminary trials, untreated grains with 100 hatched eggs had 98 live larvae on dissection of infested grains). Treatments were replicated two or four times and larval/pupal mortality was evaluated by assessing adult emergence up to 40 days after oviposition.

2.5.2 Assessment of stage of death of fumigated C. maculatus *larvae/pupae*

X-ray radiographs of fumigated and unfumigated cowpea seeds infested with appropriately aged larvae/pupae (in the above Section 2.5.1) were taken 5–6 h before fumigation (at lethal concentrations above LC_{50}), at seven days after fumigation, and on the 38th day after oviposition. The seeds in each replicate (two or three per treatment) were attached in rows on single-sided Sellotape acting as substitute bottom in a plastic frame. X-ray radiographs were taken using a Hewlett-Packard 4380N X-ray system (Faxitron series) with a tube voltage of 18 kV, a maximum tube current of about 3 mA and an exposure time of 5 min. Kodak industrial M X-ray film was developed and fixed immediately after exposure. The X-ray films were examined under a binocular microscope (x8), and by superimposing pairs of X-ray films for each batch of seeds, the changes in size, shape and position of each affected larva before and after fumigation were observed.

2.5.3 Tests with Sitophilus zeamais

(a) *Adults:* Fifteen one- to 10-day-old individually weighed adults (mixed sexes) per chamber were fumigated at rates from 8 to 16 μl litre^{-1}. Thirty or 45 insects were used per treatment, including controls, assessing mortality immediately after 24 h fumigation.

2.5.4 Tests with Dermestes maculatus

(a) *Adults:* Fifteen zero to 10-day-old individually weighed adults (mixed sexes) per chamber were fumigated at rates from 10 to 24 μl litre^{-1} (treatments replicated twice); assessing mortality soon after 24 h of fumigation.

(b) *Eggs:* Eggs (1–24 h old) were fumigated for 24 h or 48 h in two separate batches with limepeel or orangepeel oils at several rates (18–34 μl litre^{-1}).

The eggs of *D. maculatus*, usually concealed within the dried fish substrate, were collected for this experiment by confining twenty zero- to 15-day-old adults (mixed sexes) in plastic containers with pieces of double-thickness Kimwipe as substrate for oviposition, providing the insects with small pieces of dried fish as food and 10 drops of water per day. Eggs were deposited between the double layers of the paper from where they were transferred individually to fumigation glass chambers with a camelhair brush. After fumigation, eggs were picked up with the brush into ventiliated glass vials. Untreated control eggs were handled similarly but with no oil fumes in the chambers. Mortality was assessed by counting the number of eggs that hatched into larvae.

(c) *Larvae/pupae:* Fifteen late-stage larvae (20 to 25 days old) per chamber were fumigated for 48 h with limepeel or orangepeel oil at rates from 12 to 32 μl litre^{-1}. Each treatment was replicated twice, assessing mortality (failure to move away upon probing (soon after 48 h fumigation, and this was complemented by counting the number of successful pupations. Larvae were provided with food after fumigation.

In a similar experiment, 15 pupae per chamber were fumigated for 48 h with limepeel or orangepeel oils at rates from 16 to 40 μl litre^{-1}. Mortality was assessed by counting the number of adults that emerged from treated and untreated pupae.

2.6 Fumigant toxicity of citruspeel oil in the presence of grains of dried fish

For *C. maculatus*, 15 one- to two-day-old adults were fumigated per chamber with measured amounts of uninfested cowpea grains (5, 10, 20, 40, 80 or 160 g) using limepeel oil at rates from 6 to 44 μl litre^{-1}. Each treatment was replicated twice and mortality assessed immediately after 24 h of fumigation.

In a follow-up experiment, insects were fumigated at 10 μl litre^{-1} in the presence of similar amounts of grains as above. In this bioassay, mortality was assessed immediately after 24, 48, 72 or 96 h of fumigation. Since it was impossible to assess adult mortality without removing the grains from the fumigation chambers, concurrent experiments were run for each bioassay period.

With *D. maculatus*, 15 one- to 10-day-old adults per chamber with dried fish strips (10 g) were fumigated with limepeel oil at dosages from 150 to 500 μl litre^{-1}. Each treatment was replicated twice and mortality assessed immediately after 24 h of fumigation.

2.7 Statistics

All dose–response (mortality) data were analysed using a computer package for probit analysis which included tests for parallelism and relative potency based on accepted procedures.[8]

3 RESULTS

3.1 Effect of binding of oils on fumigation toxicity

(a) *Treatment surfaces:* The fumigant toxicity of limepeel oil deposited on a glass surface was shown to be

significantly ($P < 0{\cdot}05$) greater against *C. maculatus* adults than when the same amount of oil was deposited on filter paper (Table 1).

(b) *Volume of oil:* When greater total volumes (5 μl) of 3 + 2 mixtures of (+)-limonene or citronellal and the inactive, non-volatile *n*-decyl alcohol oil were deposited on filter paper, significantly ($P < 0{\cdot}001$) greater mortality of *C. maculatus* adults occurred compared with the corresponding active component alone in a constant volume (3 μl) (Table 2).

3.2 Fumigant action of citruspeel oil against adult and immature stages of test insects

3.2.1 *Tests with* Callosobruchus maculatus

(a) *Adults:* There was no significant difference between the fumigant toxicity based on 24-h LC_{50} values (no overlaps in 95% C.L.) of six different citrus oils against *C. maculatus* (Table 3). Joint probit analysis showed the data set did not contradict the hypothesis of parallelism.

(*b*) *Immature stages:* Eggs, usually laid on top of cowpea seeds, larvae and pupae of *C. maculatus* which live and feed inside the grains were all susceptible to citrus oil vapour (Tables 4 and 5). Furthermore, examination of X-ray radiographs showed that *C. maculatus* larvae which died had not developed further (size and position within seeds unchanged) than the stage at which they were 5–6 h before fumigation. The second set of X-ray films taken seven days after fumigation showed some degree of shrinkage due to water loss from affected larvae which was also indicative of rapid mortality.

Relative tolerances based on fumigant LC_{50} values (95% C.L.) for limepeel oil showed that *C. maculatus* adults, eggs and early larvae were comparable, being significantly (no overlaps in 95% C.L.) less tolerant than the late larvae/pupae (see Tables 3–5). The fumigant toxicity of limepeel oil (LC_{50}) was similar to that of the other citrus oils tested against eggs and early-stage larvae.

3.2.2 *Tests with* Sitophilus zeamais

(a) *Adults:* There was no significant differences (overlaps in 95% C.L. of LC_{50} values) between the fumigant toxicity of the six citrus oils tested against *S. zeamais*, and

TABLE 1
Relative Fumigant Toxicity of Limepeel Oil applied on Glass and Filter Paper Surfaces against *Callosobruchus maculatus* Adults

Treatment (6 μl litre^{-1})	*24-h Mortality* ($n = 60$)[ab]
Control	0
Glass	32
Filter paper	5***

[a] Four replicates (15 insects per replicate).
[b] *** Significantly different from glass at $P < 0{\cdot}001$ (2 × 2 contingency Chi-square test).

TABLE 2
Fumigant Toxicity of Pure (+)-limonene and Citronellal and Their Respective 3 + 2 Mixtures with *n*-Decyl Alcohol against *Callosobruchus maculatus* Adults

Treatment (μl litre^{-1})	*24-h Mortality* ($n = 60$)[ab]
(+)-limonene (6)	4***
(+)-limonene + *n*-decyl alcohol (3 + 2) (10)[c]	18
Citronellal (6)	24***
Citronellal + *n*-decyl (10)[c] alcohol (3 + 2)	60
Control	0

[a] Four replicates (15 insects per replicate).
[b] *** Significantly different from corresponding 3 + 2 mixture at $P < 0{\cdot}001$ (2 × 2 contingency Chi-square test).
[c] 10 μl litre^{-1} with 6 μl AI litre^{-1}.

TABLE 3
Fumigant Toxicity of Citrus Oils against *Callosobruchus maculatus* Adults[a]

Treatment	LC_{50} (95% CL)	LC_{95} (95% CL)	*Slopes* (±*SE*)[b]	χ^2	*DF*	*TF*[c]
Limepeel oil	7·99 (7·66–8·32)	10·49 (9·81–11·7)	13·88 (±1·91)	0·68	1	1
Tangerine peel oil	7·85 (7·47–8·23)	10·90 (10·04–12·53)	11·53 (±1·69)	0·71	1	1·02
Lemonpeel oil	7·77 (7·45–8·08)	10·06 (9·46–11·09)	14·67 (±1·96)	0·32	1	1·03
Grapefruit peel oil	8·22 (7·91–8·54)	10·44 (9·83–11·51)	15·82 ± 2·17	6·34	3	0·97
Orangepeel oil	8·17 (7·82–8·54)	10·95 (10·15–12·41)	12·96 (±1·85)	1·56	1	0·98
Mandarinpeel oil	8·21 (7·85–8·60)	11·16 (10·3–12·79)	12·35 (±1·80)	2·68	1	0·98

[a] $\bar{x}$ Adult weight = 4·1 (±0·2) mg (n = 50).
[b] Data do not contradict the hypothesis of parallelism.
[c] Toxicity factor relative to limepeel oil.

joint probit analysis showed the data set did not contradict the hypothesis of parallelism (Table 6).

3.2.3 Tests with Dermestes maculatus

(a) *Adults:* Most of the citrus oils tested showed similar action (based on LC_{50} values) against *D. maculatus* (Table 7). The relative toxicity of limepeel oil (as a typical citruspeel oil) to adults of the three test species as given in Tables 3, 6 and 7, shows that the toxicity (LC_{50}) of the oil against *C maculatus* adults was significantly greater (no overlapping 95% C.L.) than against either *S. zeamais* or *D. maculatus* (joint probit analysis shows that the three log-dose response curves were parallel).

TABLE 4
Fumigant Toxicity of Citrus Oils to Eggs of *Callosobruchus maculatus*, 24 h, μl litre^{-1}

Treatment	LC_{50} (95% CL)	LC_{95} (95% CL)	*Slope* (±*SE*)[a]	χ^2	*DF*	*TF*[b]
Limepeel oil	7·77 (7·11–8·50)	12·94 (10·66–15·68)	7·46 (±1·68)	13·57	4	1
Tangerine peel oil	6·62 (5·88–7·31)	14·70 (12·07–21·27)	4·7 (±0·80)	3·65	4	1·2
Orangepeel oil	6·60 (5·86–7·34)	14·39 (11·85–20·35)	4·86 (±0·77)	0·36	3	1·2
Grapefruit peel oil	8·74 (7·63–10·04)	16·19 (9·53–27·50)	6·16 (±2·24)	0·01	1	0·89
Lemonpeel oil	8·13 (7·57–8·73)	11·46 (9·35–14·04)	11·04 (±3·17)	0·02	1	0·96
Mandarinpeel oil	7·61 (6·84–8·13)	12·02 (10·92–14·43)	8·27 (±1·47)	5·27	2	1·02

[a] Data contradict the hypothesis of parallelism.
[b] Toxicity factor relative to limepeel oil.

TABLE 5
Fumigant Toxicity of Citrus Oils to Early Larvae and Late-Stage Larvae/Pupae of *Callosobruchus maculatus* within Cowpea Grains, 24 h μl litre^{-1}

Treatment	LC_{50} (95% CL)	LC_{95} (95% CL)	*Slopes* (±*SE*)	χ^2	*DF*	*TF*[a]
Early Larvae[b]						
Limepeel oil	9·06 (8·14–9·96)	18·96 (15·89–25·81)	5·13 (±0·78)	0·99	2	1
Orangepeel oil	9·47 (8·43–10·41)	17·08 (15·00–20·92)	6·42 (±0·89)	1·02	1	0·97
Tangerinepeel oil	10·21 (8·53–11·38)	17·46 (15·60–21·10	7·06 (±1·25)	0·29	2	0·93
Late larvae/pupae[b]						
Limepeel oil	17·81 (15·10–20·82)	52·18 (38·90–101·5)	3·46 (±0·65)	1·35	2	1
Orangepeel oil	12·37 (10·53–13·82)	27·83 (22·58–43·47)	4·67 (±0·93)	0·50	1	1·50
Tangerinepeel oil	11.72 (9·17–13·64)	38·33 (28·75–71·25)	3·20 (±0·63)	1·06	2	1·48

[a] Toxicity factor relative to limepeel oil.
[b] Data sets do not contradict the hypothesis of parallelism.

TABLE 6
Fumigant Toxicity of Citrus Oils against *Sitophilus zeamais* Adults,[a] 24 h, μl litre^{-1}

Treatment	LC_{50} (95% CL)	LC_{95} (95% CL)	*Slopes* (± *SE*)[b]	χ^2	*DF*	*TF*[c]
Limepeel oil	11·75 (11·25–12·25)	16·16 (15·12–17·81)	11·88 (±1·3)	0·94	1	1
Tangerinepeel oil	12·03 (11·56)–12·51)	16·01 (15·09–17·51)	13·81 (±1·4)	0·11	1	0·98
Orangepeel oil	11·44 (10·91–11·97)	16·41 (15·23–18·32)	10·50 (±1·2)	0·76	1	1·02
Lemonpeel oil	12·56 (12·07–13·05)	16·1 (15·18–17·65)	15·27 (±1·9)	0·54	2	0·93
Grapefruitpeel oil	13·41 (12·84–14·06)	18·08 (16·68–20·73)	12·69 (±1·8)	4·46	2	0·88
Mandarinpeel oil	12·89 (12·19–13·67)	19·3 (17·25–23·82)	9·39 (±1·6)	4·26	2	0·91

[a] x̄ Adult weight = 2·7 (±0·1) mg (n = 49).
[b] Data do not contradict the hypothesis of parallelism. [c] Toxicity factor relative to limepeel oil.

(b) *Immature stages:* Eggs, larvae and pupae of *D. maculatus* were shown to be susceptible to the vapour of citruspeel oils (Tables 8 and 9). The late-stage larvae and pupae were significantly more tolerant than eggs to limepeel oil fumes (48-h LC_{50}—no overlap in 95% C.L.; see Tables 8 and 9). Limepeel oil fumigant toxicity levels (LC_{50}) were again similar to those of other citrus oils against egg and pupal stages only. *D maculatus* adults were significantly more susceptible (based on 24-h LC_{50}, no overlaps in 95 C.L.) than eggs (Tables 7 and 8).

3.3 Fumigant toxicity of limepeel oil in the presence of grains or dried fish

With grains or dried fish, the fumigant toxicity of limepeel oil against *C. maculatus* or *D. maculatus* adults respectively was reduced (Tables 10 and 11). For example, the presence of 10 g of dried fish reduced the fumigant toxicity (LC_{50}) of limepeel oil against *D. maculatus* 19-fold, while 160 g of cowpea reduced the toxicity of limepeel oil vapour against *C. maculatus* by approximately 4-fold (Tables 10, 11). In the experiment with *C.*

TABLE 7
Fumigant Toxicity of Citrus Oils against *Dermestes maculatus* Adults[a] 24 h, μl litre^{-1}

Treatment	LC_{50} (95% CL)	LC_{95} (95% CL)	*Slope* (±*SE*)[b]	χ^2	*DF*	*TF*[c]
Limepeel oil	12·72 (11·63–13·64)	19·60 (17·78–22·97)	8·76 (±1·30)	8·76	1	1
Tangerinepeel oil	11·30 (9·51–12·57)	21·50 (18·75–27·73)	5·89 (±1·06)	0·13	1	1·23
Orangepeel oil	12·35 (10·89–13·50)	22·06 (19·43–27·54)	6·53 (±1·07)	0·77	1	1·30
Lemonpeel oil	16·56 (15·24–18·46)	28·59 (23·72–42·85)	6·94 (±1·37)	0·60	1	1·22
Grapefruitpeel oil	15·58 (14·19–17·36)	28·82 (23·56–45·18)	6·16 (±1·26)	2·25	1	0·91
Mandarinpeel oil	15·16 (13·84–16·74)	27·36 (22·75–40·62)	6·43 (±1·26)	0·15	1	0·98

[a] x̄ Adult weight = 20·4 (±0·6) mg (n = 40).
[b] Data do not contradict the hypothesis of parallelism.
[c] Toxicity factor relative to limepeel oil.

TABLE 8
Fumigant Toxicity of Citrus Oils to *Dermestes maculatus* Eggs, μl litre^{-1}

Treatment (*h*)[a]	LC_{50} (95% CL)	LC_{95} (95% CL)	*Slope* (*SE*)[b]	χ^2	*DF*	*TF*[c]
Limepeel oil (24)	21·46 (17·94–23·77)	39·43 (33·31–59·76)	6·23 (±1·46)	0·11	1	1
Orangepeel oil (24)	21·58 (16·63–24·24)	44·78 (34·44–101·7)	5·19 (±1·53)	0·22	1	0
Limepeel oil (48)	14·65 (11·35–16·53)	28·05 (22·42–65·67)	5·83 (±1·81)	0·13	1	1
Orangepeel oil (48)	14·87 (12·10–16·20)	23·30 (19·90 43·70)	8·48 (±2·73)	0·84	1	1·0

[a] Fumigation time before mortality assessment.
[b] 24- and 48-h data sets do not contradict the hypothesis of parallelism.
[c] Toxicity factor relative to limepeel oil at 24 or 48 h.

TABLE 9
Fumigant Toxicity of Citrus Oils to Late Larvae and Pupae of *Dermestes maculatus*, 48 h, μl litre^{-1}

Treatment	LC_{50} (95% CL)	LC_{95} (95% CL)	*Slope* ($\pm SE$)	χ^2	*DF*	*TF*[a]
Late larvae[b]						
Limepeel oil	23·14 (20·19–27·04)	64·40 (46·71–129·6)	3·70 (±0·73)	0·78	2	1
Orangepeel oil	16·53 (13·72–18·99)	42·52 (32·34–80·28)	4·01 (±0·86)	1·50	2	1·40
Pupae[c]						
Limepeel oil	23·95 (20·74–26·52)	47·89 (41·31–61·88)	5·47 (±0·87)	0·26	2	1
Orangepeel oil	19·56 (15·72–22·38)	51·15 (40·47–84·50)	3·94 (±0·79)	1·46	2	1·14

[a] Toxicity factor relative to limepeel oil.
[b,c] Data sets do not contradict the hypothesis of parallelism.

maculatus, the fumigant toxicity did not decrease proportionately with increasing amounts of grain (Fig. 1). In a follow-up series of experiments, it was shown that mortality tended to increase with time when *C. maculatus* adults were exposed to limepeel oil vapour in the presence of varying amounts of cowpea (Fig. 2).

4 DISCUSSION

In simple fumigation chambers, liquid dosing is generally on a piece of filter paper in order to increase the rate of evaporation although it is possible that absorption of liquids on cellulose fibres may affect the dose in such cases.[6] In the present work, experiments in which varying volumes of oils were applied on filter paper, or where their toxicities on glass and filter paper were compared, suggested that a proportion of the oils did in fact remain bound (not available for bioactivity). The effect of this would be to distort the dosage scale and this may have reduced the response of the test insects in this series of vapour-toxicity experiments. Therefore, although the data obtained indicate the relative differences in toxicity between oils and oil components, and this is reproducible under standardised conditions, the reported vapour-toxicity levels (LC_{50} values) are not

TABLE 10
Fumigant Toxicity of Limepeel Oil against *Callosobruchus maculatus* in the Presence of Varying Amounts of Cowpea Grain

Treatment (*amount of grains* (*g*))	*24-h Mortality* (95% CL)					
	LC_{50} (μl litre^{-1})	LC_{95}(μl litre^{-1})	*Slope* ($\pm SE$)[a]	χ^2	*DF*	*TF*[b]
Limepeel oil (0)	7·99 (7·66–8·32)	10·49 (9·81–11·70)	13·88 (±1·9)	0·68	1	4·0
Limepeel oil (5)	9·46 (9·19–9·73)	11·13 (10·70–11·84)	23·36 (±5·6)	5·61	3	3·4
Limepeel oil (10)	9·95 (9·62–10·29)	12·21 (11·47)–12·98)	18·54 (±2·9)	4·54	3	3·2
Limepeel oil (20)	10·57 (10·20–10·92)	12·70 (12·13–13·58)	20·64 (±2·6)	2·48	3	3·0
Limepeel oil (40)	11·55 (10·94–12·08)	16·23 (14·75–19·95)	11·13 (±2·2)	0·85	3	2·8
Limepeel oil (80)	19·71 (18·55–20·91)	29·91 (26·78–36·95)	9·09 (±1·5)	(2·5)	1	1·6
Limepeel oil (160)	31·95 (30·20–33·80)	48·98 (44·19–58·00)	8·87 (±1·2)	2·10	2	1·0

[a] Data contradict the hypothesis of parallelism.
[b] Toxicity factor relative to limepeel oil—160 g grains.

TABLE 11
Fumigant Toxicity of Limepeel Oil against *Dermestes maculatus* Adults in the Presence of Dried Fish

Treatment (*amount of fish* (*g*))	*24 h Mortality* (95% CL)					
	LC_{50} (μl litre^{-1})	LV_{95} (μl litre^{-1})	*Slope* ($\pm SE$)	χ^2	*DF*	*TF*[a]
Limepeel oil (0)	12·72 (11·63–13·69)	19·60 (17·78–22·97)	8·76 (±1·3)	1·61	1	19
Limepeel oil (10)	247·00 (214·3–274·5)	565·5 (472·9–774·6)	4·57 (±0·7)	4·20	2	1

[a] Toxicity factor relative to limepeel–10 g fish.

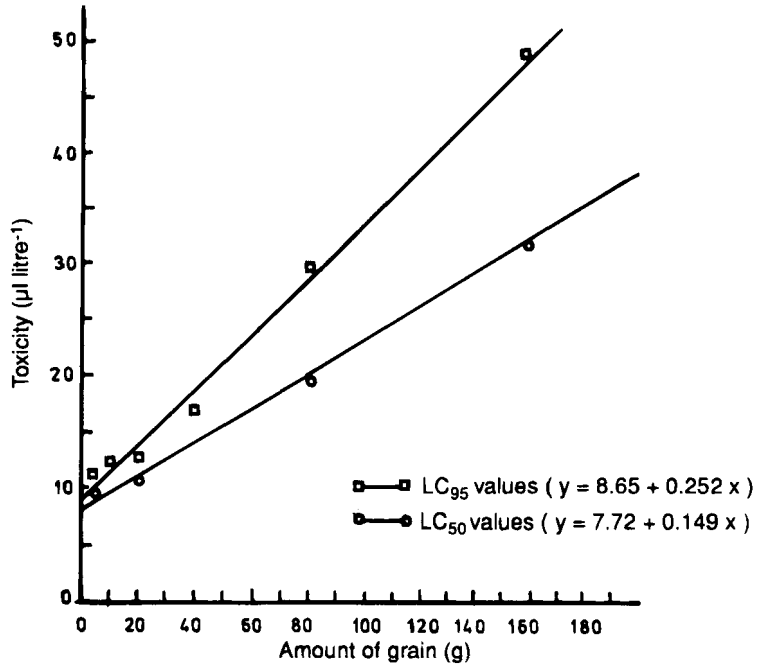

Fig. 1. Toxicity of limepeel oil vapour against *Callosobruchus maculatus* in the presence of cowpea grain.

absolute measurements but rather are numerical overestimates (underestimates with respect to levels of toxicity). This was preferred in order to present more conservative results data at this early stage of testing and measuring fumigant toxicity; secondly, dependence on glass surfaces for oil application was considered too cumbersome to operate when so many bioassays needed to be done at this primary screening stage.

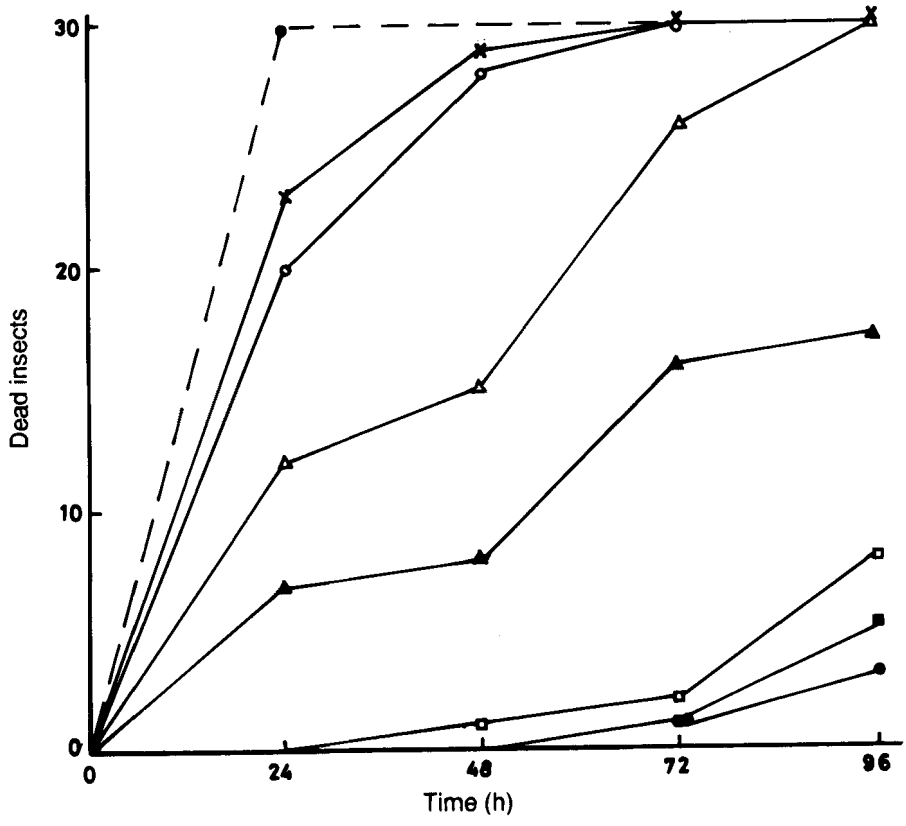

Fig. 2. Mortality of adult *Callosobruchus maculatus* exposed to limepeel oil (10 µl litre^{-1}) vapour in the presence of varying amounts of cowpea grains: (×) 5, (○) 10, (△) 20, (▲) 40, (□) 80 (■) 160 g, (●) control *C maculatus* (untreated), ((--●--) Treated *C. maculatus* (no grains)

The present study showed that all the citruspeel oils tested were of a similar order of vapour toxicity against each insect species. The similar toxicity of different citrus oils would appear to be related to the similar range of mostly terpenoid compounds[9] that constitute the oils. Joint probit analysis for the six citrus oils tested for vapour toxicity against each insect species showed that none of the three data sets contradicted the hypothesis of parallelism. Some authors[10–12] have used similar findings to support the likelihood of such compounds exerting similar biological action. However, more recently it has been shown that parallelism is not a criterion for similarity of action,[13,14] which can be extended to mean similar biological action, viz. penetration, site of action, metabolism, etc. Therefore, parallelism only means that the log tolerance distribution for the two or more compounds has the same variance. This does not contradict similar action where it exists but offers no proof of similarity and only more biological and biochemical investigations can substantiate or reject this hypothesis.

Fumigant toxicity (LC_{50}) of limepeel oil against *D. maculatus* was similar to that against *S. zeamais* adults and only slightly less (1·6 times) than that against *C. maculatus*. On the basis of adult weight, *D. maculatus* was the largest species examined with a mean weight 5 times and 7·7 times greater than that of *C. maculatus* and *S. zeamais* respectively. It therefore appears that size had little effect on susceptibility to the vapour action of citrus oils.

The fumigant toxicity of citruspeel oils extends beyond beetle pests of storage, as the vapour of these oils was found to be similarly toxic to the American cockroach, *Periplaneta americana* L. (24-h LC_{50} 18·7 μl litre^{-1}) and to house flies, *Musca domestica* L. (24-h LC_{50} 3·05 μl litre^{-1}; K. N. Don-Pedro, unpublished), indicating a broad spectrum of insecticidal action. Some authors,[15] working with a non-citrus essential oil (pheasant pepper seed oil) demonstrated vapour toxicity to a bruchid beetle (*Bruchus rufimanus* Boh.) at levels (LC_{50} 59·6 μl litre^{-1}) near to those presented in the present work. The present work has thus unequivocally established the fumigant toxicity of citruspeel oils against insects, the data presented here being the first known to the author that show significant vapour ovicidal, larvicidal and adulticidal action of citrus oils against insect stages inside and outside grains. The two previous reports[16,17] that referred to vapour toxicity of citrus oils gave no substantial experimental evidence.

Eggs and larvae that died from citrus oil fumigation appeared dried up and brittle, with some eggs (of *D. maculatus*, in particular) appearing dark brownish and shrivelled. These observations were suggestive of water loss. The demonstrated activity of citrus oils against immature stages is indirectly supported by a recent review[18] where it was stated that citrus plants were resistant to lephritid fruit fly infestation because the citrus oils had contact toxicity to eggs and larvae.

The fumigant activity of oils against *C. maculatus* larvae within cowpea grains was particularly interesting. Earlier work applying such oils directly onto infested cowpeas was shown to kill larvae but required much larger dosages (e.g. LC_{50} 1·32 ml limepeel oil per kg cowpea; K. N. Don-Pedro, unpublished). Additionally, in preliminary trials carried out by the author *S. zeamais* larvae within wheat grains were also shown to be susceptible to the toxic vapour of citrus oils at concentrations less than 25 μl litre^{-1} (1·5 × LC_{95} against *S. zeamais* adults).

X-ray studies revealed that larval stages within grains died soon after they were exposed to lethal concentrations of oil vapour, suggesting a similar response to that of adults. This rapid action is advantageous, since fumigation with citrus oils will kill the larvae rapidly which means immediate cessation of feeding activity and grain damage in storage. The active *D. maculatus* larvae and immobile pupae also died rapidly, with no appreciable development after treatment. On the other hand, larvae that received only sub-lethal doses appeared to have developed into physically normal adults.

The relative susceptibilities of different larval instars of test species were not investigated in detail but the general trend was that eggs were more susceptible than early-stage larvae, and late-stage larvae/pupae were the most tolerant to citrus oil vapour. However, it is noteworthy that these changes in susceptibility for all stages, including adults, based on LC_{50} values were not dramatic for either *C. macultus* or *D. maculatus*. This suggests that the single effective rate that needs to be worked out for practical fumigation will depend more on factors like temperature, commodity treated and species rather than on life stages which have been shown to have similar levels of tolerance for a species. Larval instars and/or weight are known to significantly affect tolerance to many synthetic insecticides.[19] For example, a direct relationship was observed between toxic doses of DDT or malathion and body weight of progressive larval instars of Mamestra brassicae (L.).[19]

Volatile components of oils may be taken up by solids during fumigation so that there may be a substantial fall in the concentration in the vapour phase within chamber free space. This phenomenon is described as sorption. Sorption is due to one or more of three main causes including:[8]

1. adsorption;
2. absorption or solution; and
3. chemical reaction.

Results of preliminary bioassays in this work showed that substantial sorption of citrus oil vapour occurred in the presence of grains or strips of dried fish and that this tended to remove some vapour and therefore longer exposures will be necessary for biological action.

However, this effect did not increase proportionally with increasing amounts of grain. Dried fish muscle appeared to be considerably more sorptive of citrus oil vapour than were grains. The fish strips being oily themselves will readily absorb the citrus oil vapour used here.

There was also an observed tendency for adult mortality (in spaces between and above grains) to increase with time in the presence of cowpea grains. This, added to the fact that *C. maculatus* larval stages that live within cowpea seeds were killed easily by citrus oil vapour, suggests that citrus oil vapour does not enter into chemical reaction with, or remain permanently bound to, the grain material externally or internally. Although absorption and adsorption of the citrus vapour may be taking place, the interesting point was that sufficient vapour reaches the larval stages inside the seeds to kill them.

There was some experimental evidence collected in trials (K. N. Don-Pedro, unpublished) which showed that longer exposure periods in fumigation experiments with citrus oils led to higher mortality, as expected. However, for convenience, a 24-h fumigation period was adopted in most experiments, at the end of which mortality was assessed. Therefore end-point mortality (post-fumigation mortality) was not determined, deferring it to future work. It is suggested that future work should investigate how the vapour toxicity of citrus oils or that of their most active component is governed by the CT or Haber's rule in elaborate fumigation chambers. This may be achieved in part through bioassays in which time–response (mortality) relationships under varying dosages for a target species are studied. Additionally, sorption, for more practical storage systems, is usually measured by treating a larger quantity of commodity than in this initial study; e.g. a sack of commodity in a proper fumigation chamber, and measuring the fall in concentration (within free space), possibly employing sophisticated analysis by gas chromatography. Such elaborate experiments, although expensive, should determine more accurately the practicality of employing citruspeel oils or better still, a single most active component, as fumigant in a limited or wide-scale storage pest control system.

It is concluded that the data presented so far show that citrus oils are sources of biologically active vapour that are potentially useful insecticides. Further investigation of the fumigant properties (particularly chemical and physical) of these natural products is desirable. This is especially so because preliminary experiments in this work have demonstrated the sorptive nature of the insecticidal citrus oil vapour, a property that may hinder their being practical fumigants for large-scale treatments. However, the possiblities of employing these natural fumigants from oils that are pressed from peels of citrus fruits (easily available in the tropics) in small-scale traditional storage systems may be worthy of proper investigation. In this regard a series of experiments is planned to verify the maximum quantity of grains that can be efficiently disinfested with citrus oil vapour. For example, varying quantities (5–80 kg) of grains will be fumigated in closed traditional earthen pots and or cribs with multiple bamboo shelves (dosing the content of each shelf separately from oils in open glass dishes) covered with tarpaulin (to make the crib semi-gas tight) for varying periods. Secondly, the work so far has indicated that the use of a complex mixture of chemicals such as citrus oil as a fumigant may present problems of different rates of sorption of components, and, therefore, accurate prediction of toxicity levels in practical situations may be impossible. These problems may be due to possible joint actions of constituents against a background of different rates of release of the components, which in turn depends on how they are presented and on which substrate. Therefore it may be better to use a single, most volatile, constituent which would be more predictable but more expensive to produce. In spite of current problems and high cost of research, the search for new fumigants with the correct physical, chemical and toxicological characteristics originating from synthetic or natural products is considered necessary and justifiable, mainly because the effectiveness of the most common synthetic fumigant (phosphine)[6] in the market is now significantly limited in Nigeria by widespread storage pest resistance, high cost and frequent use of high concentrations in small- and large-scale commodity stores leading to an increased number of cases of accidental human poisoning.

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